Title: Salivary Biomarkers and Training Load during Training and Competition in Paralympic Swimmers

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Abstract

Purpose: Stress responses in athletes can be attributed to training and also competition, where increased physiological and psychological stress may negatively impact on performance and recovery. The aim of this study was to examine the relationship between training load and salivary biomarkers IgA, alpha-amylase (AA) and cortisol across a 16-week preparation phase and 10-day competition phase in Paralympic swimmers. ***Methods:*** Four Paralympic swimmers provided bi-weekly saliva samples during three training phases – 1) normal training, 2) intensified training and 3) taper as well as daily saliva samples in the 10 day Paralympic competition (2016 Paralympic Games). Training load (TL) was measured using session-RPE. ***Results:*** Multi-level analysis identified a significant increase in sIgA (94.98 (27.69) $\mu g.ml^{-1}$), sAA (45.78 (19.07) $\mu g.ml^{-1}$) and salivary cortisol (7.92 (2.17) ng.ml) during intensified training concurrent with a 38.3% increase in TL. During taper phase, a 49.5% decrease in TL from the intensified training phase resulted in decrease in sIgA, sAA and salivary cortisol; however, all three remained higher than baseline levels. A further significant increase was observed during competition in sIgA (168.69(24.19) $\mu g.ml^{-1}$), sAA (35.86(16.67) $\mu g.ml^{-1}$) and salivary cortisol (10.49(1.89) ng.ml) despite a continued decrease (77.8%) in TL from taper phase. ***Conclusions:*** Results demonstrate performance in major competition such as Paralympic Games despite a noticeable reduction in TL induces a stress response in athletes. Due to elevated stress response observed, modifications to individual post-race recovery protocols may be required to enable athletes to maximise performance across all ten days of competition.

Keywords: Paralympic Games, Stress Response, Salivary Cortisol, Salivary Immunoglobulin A, Salivary Alpha-Amylase

Introduction

Athletic training is based on the principle of progressive overload where increased training stressors combined with appropriate recovery are employed to produce a positive training adaptation.[1] Included in a periodised pre-competition preparation plan is a period of intensified training followed by a taper phase where training volume is typically reduced whilst maintaining or even increasing intensity resulting in positive adaptation and performance enhancements.[2] However, responses to athletic stress are highly individualised with athletes recovering from the same training stimulus differently.[3] Whilst the taper period is designed to reduce training stress and promote recovery, performance in athletic competition has been shown to induce a psychophysiological stress response irrespective of the reduction in training load (TL). Given the sensitivity of immune function to physiological and psychological stressors, immune and stress salivary biomarkers may assist in monitoring the athletic responses to training and competition demands.[4]

Training load monitoring can be used to measure the individual training stress for each athlete using physiological and psychological variables, ensuring individualisation of training prescription and minimising risk of overtraining.[5] Originally proposed by Foster et al., [6] the session rate of perceived exertion (sRPE) method quantifies internal training load as an arbitrary unit using an athletes RPE score multiplied with training session duration in minutes. The sRPE method is sufficiently accurate to measure training session intensity if HR data is not available or a more practical method is required for calculating training load.[7] It has also been shown to be a reliable method of quantifying training load in water based sports where heart rate is not easy collected having previously been validated in swimmers.[8]

Salivary biomarkers are easily accessible and non-invasive measures which can be quantified quickly and repeatedly.[9] Saliva contains both immunity and stress biomarkers including immunoglobulin A (IgA), alpha-amylase (sAA) and cortisol, all of which have been shown to respond to training and competition stress in athletes. IgA secreted in saliva, has a primary role in defence against infection of the upper respiratory tract, and has been established as a reliable biomarker for identifying risk of infection in elite athletes.[10] Previous research has reported an inverse relationship between salivary IgA levels and incidence of illness in athletes[11], while changes in salivary IgA levels may also indicate periods of excessive training or inadequate recovery.[12] Salivary alpha-amylase (sAA) produced in the salivary glands has been shown

to be a reliable indicator of the response of the sympathetic nervous system to exercise.[13] This response appears to peak rapidly at the onset of a stressor before returning to baseline levels 30-60 minutes later[14] and this acute response has been associated with both physical and psychological stressors.[15] Salivary cortisol is secreted by the adrenal cortex in response to physical or psychological stress, and can provide a reference for cortisol levels in the blood with research showing more pronounced changes in saliva in response to exercise.[9] Cortisol levels have been shown to increase concurrently with training load in swimmers[16] while in rugby union players, increases were observed following an international level game and remained elevated above pre-game levels fourteen hours later.[17] Regular monitoring of controlled resting levels of salivary biomarkers has been recommended to determine individual reference data as variations within and between subject groups implies that the stress response to training load, competition and additional external stressors is highly individual.[10]

Despite the shift in focus from rehabilitative participation to elite level sport, research into Paralympic sport has lagged behind the large body of scientific investigation of able-bodied athletes. Training load and athletic response must be monitored in a bid to fully understand the training and recovery needs of this highly individual athletic population. Therefore the aim of this study is to examine the training loads and salivary biomarker responses during preparation and competition in four Paralympic swimmers.

Methods

Participants

Four elite Paralympic swimmers (1 male, 3 female, age 19 ± 4yrs, body mass 48.5 ± 7.6kg) selected for competing at Rio 2016 Paralympic Games participated in this study. Details of individual training age, impairment type and swimming classification are presented in Table 1. A typical training week consisted of seven to nine pool sessions of two hour duration each (14-18 hours weekly) and two gym sessions of one hour duration (2 hours weekly). Individual swimming programs were prescribed dependent upon swimming class; with higher classed swimmers completing the higher training hours. The athletes had been competing regularly in international competitions for at least 3 years. All four athletes had competed and reached finals in the World Championships in the previous 12 months. Testing protocols formed part of the on-going physiological support programme which swimmers were familiar with before participation in this study. All participants were fully informed of the requirements and potential risks and benefits of participating with a written informed consent completed before commencement of data collection. All

experimental procedures were approved by University of Limerick Ethics Committee.

Experimental Design

Athletes were monitored throughout a twelve month period in the run-up to the 2016 Paralympic Games. Four periods of collection were established in the 16 weeks before the Paralympic Games: 1) a baseline non-competition period of 4 weeks (11 samples), 2) an intensified training period of 2 weeks (6 samples), 3) a taper of 10 days (4 samples) and 4) a competition period of 10 days (10 samples). No samples were collected during the first seven days upon arrival in Brazil in order to reduce any impact of travel fatigue and jet lag on salivary biomarker response. During the non-competition periods, salivary data was collected twice weekly to determine a baseline hormonal profile whilst daily samples were made each morning during the Paralympic Games competition, reflecting both race and resting day measures, to depict the salivary hormone response when competition stress would be highest.

Data Collection

Salivary Biomarkers. Saliva samples were collected in the morning, 30 minutes after waking, before breakfast and before any physical exercise had been undertaken. Sampling was kept to a consistent one hour time block for each athlete to minimise impact of circadian variation on salivary biomarkers. Swimmers were instructed not to brush their teeth before providing the saliva sample. Salivary samples were collected using an IPRO (Soma Bioscience, Wallingford, UK) oral fluid collector (OFC) kits. The ease of sample collection using the IPRO OFC kits allowed athletes to collect their saliva sample at home. The sampling protocol was followed in accordance with manufacturer's guidelines. The OFC is placed in the mouth and collects 0.5mL of saliva in one sample. A volume indicator within the swab handle changed colour to indicate when sufficient saliva volume has been collected. The swab was then removed from the mouth and placed into the IPRO OFC buffer. The duration of collection time was less than 60s. The buffer contains extraction agents to draw the target analytes from the swab into the buffer. Samples were analysed using an IPRO lateral flow device (LFD) with separate cartridges used to analyse IgA/AA and cortisol. The LFD has previously been validated against ELISA analysis ($r = 0.89$, $p < 0.01$ and CV = 9.4%).[15] Two drops of buffer mix from the collector kit were added to the sample window on the LFD cartridges. After a standing time of 10 minutes, sample intensity is measured in an IPRO LFD reader and a quantitative value given.

Training Load. The session-RPE method was used to calculate training load as proposed by Foster et al.[6] Fifteen minutes after every training session[19] swimmers were asked to rate the intensity of the session using the CR-10 RPE scale.[20] The total session duration including warm-up and cool-down was recorded in minutes and multiplied by the RPE score given by each athlete (training load = duration x intensity). Training load is expressed in arbitrary units (AU).

Statistical Analysis

Mean and standard error were calculated for training load, salivary IgA, alpha-amylase and cortisol levels collected during the four training phases. Data was analysed using multilevel modelling approach using Multilevel Models Project MLn[21] to investigate longitudinal (repeated measures) data. Multilevel analysis is an extension of multiple regression. A random intercept model with 2 levels was created for IgA, AA and cortisol separately – time (level 1) nested within athlete (level 2). Analysis was used to identify changes in mean values of three salivary biomarkers across the four identified time periods.

Results

Figure 1 shows mean ± SE values for training load and salivary biomarker levels at each training phase (1 = baseline, 2 = intensified training, 3 = taper, 4 = competition).

The multi-level analysis (Table 2) identified a significant increase in levels of sIgA (94.98 (27.67) $\mu g.ml^{-1}$), sAA (45.88 (19.07) $\mu g.ml^{-1}$) and salivary cortisol (7.92 (2.17) ng.ml) from baseline to intensified training. Increases were concurrent with a 38.3% increase in training load during this period.

During taper phase, a 49.5% decrease in the training load from the intensified training phase resulted in a decrease of sIgA, sAA and salivary cortisol levels. However, all three biomarker levels remained higher than baseline levels.

A further significant increase from baseline was observed during competition phase in sIgA (168.69(24.19) $\mu g.ml^{-1}$), sAA (35.87(16.67) $\mu g.ml^{-1}$) and salivary cortisol (10.49(1.89) ng.ml). Increases in all three biomarkers occurred despite a continued decrease of 77.8% in training load from taper phase.

Minimal changes between rest and race day levels of sIgA (380.62 $\mu g.ml^{-1}$ vs 379.77 $\mu g.ml^{-1}$ respectively) were observed. In contrast, race day induced an acute significant increase in salivary cortisol (-7.19(2.07) ng.ml) and sAA (-55.82(17.57) $\mu g.ml^{-1}$) compared to rest day, further demonstrating an elevated stress response associated with participating in a Paralympic Games.

Discussion

The present study was designed to examine training load and the associated stress response through the measurement of three salivary biomarkers in Paralympic swimmers during training and performance in major competition. Salivary IgA, AA and cortisol were shown to respond to changes in training load across the training season. During a period of intensified training, a 38.3% increase in training load was associated with significant increases in all three salivary markers while a subsequent decline in training load of 49.5% during a taper phase coincided with decreases in sIgA, sAA and salivary cortisol. Interestingly despite a further 77.8% reduction in training load compared to the taper phase, during the Paralympic Games salivary biomarkers were significantly increased from baseline demonstrating an induced stress response in all four Paralympic swimmers.

The emergence of a validated point of care test for sIgA and sAA has allowed the quick analysis of salivary biomarkers.[22] Furthermore it has been suggested cortisol changes in response to exercise may be more pronounced in saliva compared to blood as salivary cortisol represents biologically active, free fraction of blood cortisol.[23] Thus salivary biomarkers have emerged as a popular monitoring tool in athletic populations due to the ease of use and non-invasive method of sample collection. Research suggests that athletes undertaking intensive and prolonged training may be at a higher risk of upper respiratory tract infections (URTI).[24] Representing the body's first line of defence against URTI, monitoring immune function through sIgA levels can determine the effect of exercise on mucosal immunity.[9] Longitudinal studies amongst elite endurance athletes have shown sIgA levels to decrease in response to increases in volume and duration of training, with a decline appearing to contribute to the increased risk of illness in athletes.[10] In contrast to this, findings from the current study observed a significant increase from baseline in sIgA during intensified training period correlating with a 38.3% increase in training load. In a study investigating high school basketball players, Tharp[25] reported a 25.1µg.ml^{-1} increase in mean sIgA levels across a season and suggested chronic training may result in increases in resting IgA levels providing further protection from infection risk. Supporting this Gleeson and Walsh[26] reported that moderate exercise can increase sIgA concentrations thus decreasing the risk of URTI. The The four athletes participating in the current study were 4-5 weeks away from competition during the intense training camp after a long training season and combined with sufficient recovery may explain why no decreases occurred in sIgA. Following this, a taper phase characterised by a gradual decrease in training

volume and increase in intensity resulted in a drop in overall training load and a subsequent decline in levels of sIgA. During this time declines in sAA and salivary cortisol were also observed.

Athletic competition has been shown to induce a stress response in athletes. sAA has been shown to be a reliable indicator of the adrenergic response to exercise[13] therefore can be measured alongside cortisol to depict the stress response to training and competition in a bid to optimise recovery. However, sAA has been reported to be a more sensitive measure to exercise-induced stress than cortisol as it does not require transport from blood to saliva.[27] sAA has been shown to significantly increase in response to competition with Kivlighan and Granger[14] reporting an increase of 156% during ergometer competition in male and female collegiate rowers. Findings from this study demonstrated a significant response in sAA levels during competition compared to baseline and are in line with those reported by Edmonds et al.[22] who observed a prolonged elevation in sAA following a weekend of elite level competition in disability swimmers. Furthermore Diaz et al.[28] compared sAA levels before and after a race event during competition and on a control day in swimmers and reported higher levels during competition which were attributed to increased psychological and physical stress. The increase in sAA in the current study during the competition phase can potentially be attributed to two stressors – an elevation from increased competition performance as well as the psychological stress of participating in a major competition.

Salivary cortisol has been extensively researched as an indicator of training stress. Gomes et al.[29] reported increases in training load and stress scores correlated with increases in salivary cortisol levels in tennis players during a periodised training programme before returning to baseline levels during a taper week. In line with these findings, we observed during the intensified training phase an increase in training load of 38% from baseline induced a stress response in athletes and resulted in a significant increase in salivary cortisol levels. Furthermore a decrease in salivary cortisol during the taper phase was accompanied by a decline in training load of 49.5%. However, further decreases in training load during the competition phase were not associated with additional declines in salivary cortisol levels. A study in soccer players showed a reduction in cortisol during recovery periods compared to periods of intense training.[30] The present study showed similar findings with a mean decrease in salivary cortisol levels during taper phase following an increase during the intensified training phase. In contrast salivary cortisol levels actually increased significantly again from baseline to their highest levels during the competition phase at a point where training loads were lowest.

The continued increase in salivary cortisol levels during this competition phase may be explained by an elevated psychological stress response induced by performance in a major competition such as the Paralympic Games.

A limitation of this study was the absence of a psychological assessment measure for example POMS or REST-Q to understand the stress impact on the athletes. However, similar to Moreira et al.[31] it is reasonable to suggest performance at a major international competition is a stressful situation for any athlete. A further limitation is the small sample size used in this case study, however, this accounts for the entire Paralympic swimming population of the Irish team and can therefore be regarded as being representative of the athletic population.

Practical Applications

The findings of this study suggest that coaches and support staff should recognise the stress response associated with participation in major competition despite training loads decreasing. According to Kellman[32] heightened stress levels in athletes can limit the ability to recover and require additional recovery activity. Our results indicate post-race recovery must account for not just the physiological stress on the body as a result of racing but also the individual physiological and psychological stress response to major competition. Additional recovery modalities, for example nutritional interventions, increased sleep and increased post-race swim down may be required to meet the increased recovery demand of athletes and assist in maximising performance across all ten days of competition.

Conclusion

This study aimed to examine the responses of sIgA, sAA and salivary cortisol to training and performance in competition. All three salivary markers were shown to respond to changes in training load with increases during more intense training and decreases during taper. Performance in major competition was shown to induce a further stress response in the athletes. Significant increases in sAA and salivary cortisol levels were observed during competition period compared to baseline despite low training loads. With a decrease in training load during this last phase it is reasonable to associate the response with increased psychological stress of participating in a competition as significant as a Paralympic Games.

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Figure Captions

Figure 1: Data mean ± SE. Salivary IgA ($\mu g.ml^{-1}$), salivary cortisol (ng.ml), salivary alpha-amylase ($\mu g.ml^{-1}$) and training load across the four time points (1=baseline, 2=intensified training, 3=taper, 4=competition). * indicates statistical significance between phase 1 and 2 determined by multi-level regression analysis. ** indicates statistical significance between phase 1 and 4 determined via multi-level regression analysis.

Table 1: Athlete characteristcs. [a] IPC Classification code; [b] Years competing as part of the national Paralympic swim team

Table 2: Values are means ± SE. Baseline training salivary levels were used as constant, indicated by (a) and compared to levels during three other training phases indicated by (△a). Changes from baseline in all three salivary markers were significant at intensified training phase and again during competition phase. The between-subject variances (at level 2)

were not significant but the within subject variances (at level 1) were all significant.

Table 1. Athlete characteristics

Athlete	Gender	Disability Type	Swimming Class[a]	Competition Experience (yrs)[b]
1	M	Les Autres	S5	5
2	F	Amputee	S9	9
3	F	Arthrogryposis	S8	4
4	F	Hypochondroplasia	S6	3

Table 2. The multilevel regression analysis of salivary levels for the four athletes competing at the 2016 Paralympic games.

	Salivary IgA		Salivary AA		Salivary Cortisol	
Fixed explanatory variables						
Parameter	Estimate	S. Error	Estimate	S. Error	Estimate	S. Error
Constant (a)	148.2	18.75	69.13	18.09	16.29	1.64
Intensified Training (△a)	94.98	27.67	45.88	19.07	7.92	2.17
Taper (△a)	25.58	31.62	29.73	21.79	1.97	2.48
Competition (△a)	168.7	24.19	35.87	16.67	10.49	1.89
Random Variables						
Level 2 (between athletes)						
Variance	Estimate	S. Error	Estimate	S. Error	Estimate	S. Error
	220.3	430.9	745.63	653.91	3.47	4.14
Level 1 (within athletes)						
Variance	Estimate	S. Error	Estimate	S. Error	Estimate	S. Error
	11253.38	1497.1	5341.12	710.64	69.08	9.19

Figure 1. Salivary IgA, salivary AA, salivary cortisol and training load across four training phases